



UNIVERSITI PUTRA MALAYSIA

**ENZYMATIC INCORPORATION OF OLEIC ACID INTO REFINED
BLEACHED AND DEODORISED PALM OLEIN**

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FS 2007 10

**ENZYMATIC INCORPORATION OF OLEIC ACID INTO REFINED
BLEACHED AND DEODORISED PALM OLEIN**

By

LIEW HAN-FANG

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirement for the Degree of Master of Science**

June 2007



for papa with love

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: Prof. Dr. Dzulkefly Kuang Abdullah, PhD

Faculty: Science

High oleic oils are very much in demand. Nutritionally, they are perceived to reduce cardiac related diseases. Oxidatively, they are more stable than polyunsaturated oils. However, palm olein, with natural oleic content of less than 50%, the olein solidifies in temperate climate. This study was conducted to investigate the performance of lipases on oil substrate to obtain high oleic content cooking olein.

In the first part of the work, the effect of several reaction conditions on lipase-aided acidolysis of palm olein with oleic acid was studied. Results showed no significant difference on the effect of molecular sieve added into acidolysis process. Studies on the other effects indicated that the optimal condition for *T. Lanoginosa* lipase was at 50 °C, 10 % (w/w) lipase loading and substrate concentration of 1:2 (POo:OA mole ratio). Eight hours was selected as the best reaction time. The acidolysis process also increased the slip melting point of palm olein after 8 h reaction with *T. Lanoginosa* lipase registering the largest increase (3.8 °C) compared to the initial unreacted

mixture. The catalytic stability of *T. Lanoginosa* lipase, after being subjected to ten runs of repeated usage indicated that the lipase can be reused to produce fairly constant products on a larger scale.

The rates of acidolysis were found to vary with different lipase sources. Generally both the *T. Lanoginosa* and *Alcaligenes sp.* lipases also produced the highest degree of acidolysis and % FFA, with oleic acid content up to about 60 %. Both lipase-catalysed mixtures cause the lift in slip melting point and solid fat content (SFC) at a higher temperature (above 25 °C) in all the two mixtures studied compared to the unreacted commercial olein.

The second part of this study, was studied the factors affecting the transesterification of palm olein with methyl oleate. Both lipases were used in acidolysis as a model to study the effect of temperature, lipase loading, substrate concentration and reaction time. The optimum condition for transesterification was at 50 °C, 10% (w/w) lipase load, substrate concentration of 1:2 (POo:MO mole ratio) and 2 h incubation time for both lipases. Similarly, the transesterification process also increased the SMP of palm olein after 2 h reaction with *T. Lanoginosa* lipase registering increase of 1.3 °C compared to the initial unreacted mixture. The SMP and SFC results share the similar trend with acidolysis, while the oleic acid content generated from transesterification was about 54 %, slightly lower than acidolysis.

Generally, the products obtained from interesterification contained higher oleic acid compared to that of starting POo (about 42-46%). Therefore, interesterification

process without any further treatment, has improved the unsaturation of a palm oil product as well as monounsaturation content, which is comparable to olive oil.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGGABUNGAN ASID OLEIK SECARA ENZIM KEPADA MINYAK
SAWIT DISULING DILUNTUR AND DINYAHBAU**

Oleh

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Minyak tinggi oleik adalah sangat diperlukan. Dari segi zat makanan, ia mengerti kebolehan untuk mengurangkan kemungkinan sakit jantung. Dari segi pengoksidaan, ia adalah lebih stabil daripada minyak poli-tak-tepu. Bagaimanapun, minyak sawit olein mengandungi kandungan oleik kurang daripada 50% secara semulajadi, ini menyebabkan ia senang mempejal di negara-negara bermusim. Penyelidikan ini bertujuan untuk mengetahui perlaksanaan enzim dalam bahan minyak agar mendapat minyak masak berkandungan oleik yang tinggi.

Pada bahagian pertama bagi penyelidikan ini, kesan beberapa keadaan tindak balas asidolisis telah dikaji. Keputusan tidak menunjukkan perbezaan terhadap kesan saringan molekul yang telah ditambah. Pengajian terhadap kesan lain menunjukkan, keadaan optima bagi proses asidolisis oleh enzim *T. Lanoginosa* adalah, pada suhu 50 °C, sukatan enzim 10 % (jisim/jisim) dan kepekatan bahan tindak balas 1:2 (POo:OA nisbah mol). Masa tindak balas yang paling sesuai adalah 8 jam. Proses asidolisis meningkatkan takat lebur sorong (SMP) minyak sawit olein sebanyak 3.8 °C selepas tindak balas selama 8 jam dengan enzim *T. Lanoginosa*. Kajian atas

kestabilan mungkin bagi enzim *T. Lanoginosa* sebanyak 10 kali menunjukkan bahawa, enzim tersebut boleh dikitar semula dan masih mengekalkan penghasilan yang malar.

Kadar tindak balas asidolisis adalah berbeza antara sumber-sumber enzim yang berlainan. Lazimnya, kedua-dua enzim *T. Lanoginosa* dan *Alcaligenes sp.* dapat menghasilkan darjah asidolisis and % asid lemak bebas (FFA), dengan kandungan asid oleik sebanyak 60 %. Kedua-dua campuran hasil tindak balas mengakibatkan peningkatan takat lebur sorong (SMP) dan kandungan pepejal lemak (SFC) pada suhu yang melebihi 25 °C, berbanding dengan minyak sawit olein komersial.

Bahagian kedua bagi kajian ini adalah mengkaji faktor-faktor yang mempengaruhi tindak balas transesterifikasi minyak sawit olein dengan metil oleat. Kedua-dua enzim yang terlibat dalam asidolisis digunakan sebagai model untuk mengkaji kesan suhu, sukatan enzim, kepekatan bahan dan masa tindak balas. Hasil kajian menunjukkan keadaan optima bagi kedua-dua enzim adalah 50 °C, 10 % (jisim/jisim) sukatan enzim, kepekatan bahan 1:2 (POo:MO nisbah mol) dan masa tindak balas selama 2 jam. Begitu juga, proses transesterifikasi meningkatkan takat lebur sorong (SMP) minyak sawit olein selepas bertindak balas selama 2 jam dengan enzim *T. Lanoginosa* sebanyak 1.3 °C. Keputusan takat lebur sorong (SMP) dan kandungan pepejal lemak (SFC) juga menunjukkan kecenderungan yang sama dengan asidolisis. Manakala, kandungan asid oleik yang terhasil dari transesterifikasi mencapai 54 %, agak lebih rendah daripada asidolisis.

Produk yang terdapat daripada interesterifikasi mengandung asid oleik yang lebih tinggi daripada POo pada mulanya (42-46%). Maka, interesterifikasi dapat meningkatkan ketaktepuan minyak sawit dan khasnya kandungan mono-ketaktepuan.

ACKNOWLEDGEMENTS

I wish to thank the Director-General of Malaysian Palm Oil Board for providing the graduate assistantship to pursue the degree of Master of Science in the Faculty of Science of Universiti Putra Malaysia. I wish to express my appreciation and gratitude to my supervisor, Prof. Dr. Dzulkefly Kuang Abdullah of the Department of Chemistry, Faculty of Science for his guidance, constructive suggestions throughout the course of my study. My grateful thanks also to Dr. Siew Wai Lin of MPOB, a member of my supervisory committee, for giving me an opportunity to be exposed to the many aspects of chemical, physical and technology of fats and oils, as well as constant encouragement, invaluable guidance and constructive suggestions. The suggestions and insightful comments on my research given by another member of my supervisory committee, Dr. Cheah Kien Yoo, is gratefully acknowledged.

Many thanks are also due to the following people: Pn. Noraini Mohammad, Pn. Rosnani Hassan, En. Abdullah Abdul Rahman, and Cik Suraya Abdul Rahman for their assistance and support. Special thanks also go to all faculty members, staffs, technicians, fellow graduate students (they know who they are) for their kind cooperation throughout my study and for taking the time to share their expertise, knowledge and wisdom with me.

Last but not least, I would like to thank my family for their encouragement, understanding and patience in sharing my excitements and frustrations of research.



I certify that an Examination Committee has met on 8th June 2007 to conduct the final examination of Liew Han-Fang on her Master of Science thesis entitled “Enzymatic Incorporation of Oleic Acid into Refined Bleached and Deodorised Palm Olein” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LIEW HAN-FANG

Date: 15 August 2007

TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xv
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS/NOTATIONS/GLOSSARY OF TERMS	xx

CHAPTER

1	INTRODUCTION	1
2	LITERATURE REVIEW	7
	2.1 Fats and Oils	7
	2.2 Palm Oil	9
	2.3 Lipases	12
	2.3.1 Definition of Lipases	12
	2.3.2 Properties of Lipases	15
	2.3.3 Specificity of Lipases	17
	2.4 Food Application of Lipases	19
	2.5 Interesterification of Fats	21
	2.5.1 Chemical Interesterification	22
	2.5.2 Enzymatic Interesterification	23
	2.6 Comparison of chemical and enzymatic interesterification	27
	2.7 Factors Affecting Equilibrium and Rate of Chemical and Enzymatic Interesterification	28
3	MATERIALS AND METHODS	31
	3.1 Materials	31
	3.1.1 Substrates	31
	3.1.2 Biocatalysts	31
	3.1.3 Solvents and reagents	32
	3.2 Procedures	32
	3.2.1 Acidolysis Reaction	32
	3.2.2 Interesterification Reaction	34
	3.2.3 Methyl Esters (MEs) Preparation from Triacylglycerols (TAGs)	34
	3.2.4 Purification of Methyl Esters to obtain Methyl Oleate by Fractional Distillation	35
	3.2.5 Short Path Distillation	36

3.3	Test Methods to Determine the Quality of Transesterified Products	36
3.4	Instruments	41
4	RESULTS AND DISCUSSION	43
4.1	Enzymatic Interesterification Reaction	43
4.2	Optimisation of Conditions of Interesterification	43
4.3	Acidolysis Reaction of Palm Olein with Oleic Acid	44
4.3.1	Effect of Molecular Sieve	44
4.3.2	Effect of Substrate Mole Ratio	47
4.3.3	Effect of Lipase Load	54
4.3.4	Effect of Temperature	61
4.3.5	Effect of Reaction Time	65
4.3.6	Catalytic Stability of Repeated Use of Immobilised Lipase	68
4.3.7	Effect of Different Lipases on Acidolysis	71
4.4	Transesterification Reaction of Palm Olein with Methyl Oleate	76
4.4.1	Effect of Temperature	76
4.4.2	Effect of Substrate Mole Ratio	81
4.4.3	Effect of Lipase Load	86
4.4.4	Effect of Reaction Time	93
4.4.5	Effect of Different Lipases on Transesterification	96
4.5	Comparison of Fatty Acid Composition of Interesterified Oleins with Other Liquid Oils	99
4.6	Physical and chemical properties of lipase-catalysed of Palm Olein with Oleic Acid and Methyl Oleate Mixtures	101
4.6.1	Slip Melting Point (SMP) and Solid Fat Content (SFC)	101
4.6.2	Thermal Analysis	104
5	CONCLUSION	108
5.1	Conclusion	108
5.2	Recommendations	110
	BIBLIOGRAPHY	112
	APPENDICES	128
	BIODATA OF THE AUTHOR	147

LIST OF TABLES

Table		Page
1	Main Fatty Acids in Edible Oils and Fats	10
2	Fatty Acid Composition (%) of Palm Oil by Various Authors	11
3	Fatty Acid Composition (%) of Malaysian Palm Oil	11
4	Fatty Acid composition before (control) and after acidolysis at various molecular sieve loadings	47
5	OA incorporated within time intervals (substrate mole ratio; POo:OA)	48
6	Triacylglycerol composition/Fatty acids composition and concentration of palm olein before (control) and after acidolysis at various Substrate Mole Ratio.	53
7	Changes of content within time intervals (lipase load; POo:OA)	55
8	Triacylglycerol composition/Fatty acids composition and concentration of palm olein before (control) and after acidolysis at various Lipase Loading	56
9	Changes of content within time intervals (temperature; POo:OA)	62
10	OA incorporated within time intervals (temperature: POo:OA)	63
11	Triacylglycerol composition/Fatty acids composition and concentration of palm olein before (control) and after acidolysis at various reaction temperature	64
12	Triacylglycerol composition/Fatty acids composition and concentration of palm olein before (control) and after acidolysis at various Incubation Time. Values have been reformatted to 100%	67
13	Changes of content within time intervals by Lipase PL	73
14	OA and PA changes catalysed by different lipases within time intervals	73
15	Comparison of TAG composition/Fatty acids composition and concentration of palm olein at equilibrium. Catalysed by <i>T. Lanuginosa</i> (8h) and <i>Alcaligenes sp.</i> (2h) respectively	74
16	Changes of content within time intervals (temperature; POo:MO)	76

17	Changes of content within time intervals (lipase load; POo:MO)	87
18	Triacylglycerol composition/Fatty acids composition and concentration of palm olein before (control) and after transesterification at various Lipase Loading	92
19	Triacylglycerol composition/Fatty acids composition and concentration of palm olein before (control) and after transesterification at various Incubation Time	94
20	OA increment and PA decrement catalysed by different lipases within time intervals (POo:MO)	97
21	Comparison of TAG composition/Fatty acids composition and concentration of palm olein at equilibrium. Catalysed by <i>T. Lanuginosa</i> (2 h) and <i>Alcaligenes sp.</i> (4 h) respectively	98
22	Fatty acid composition (FAC) of interesterified oleins as compared to other soft oils	100
23	Slip Melting Point (SMP) and Solid Fat Content (SFC) of interesterified Palm Olein:Oleic Acid and Pam Olein:Methyl Oleate mixtures	103
24	Comparison of TAG composition/ Fatty acids composition and concentration of palm olein after Short Path Distillation at equilibrium. Catalysed by <i>T. Lanuginosa</i> with mixtures of Palm Olein:Oleic Acid and Palm Olein:Methyl Oleate	107

LIST OF FIGURES

Figure		Page
1	Classification of Fatty Acids	9
2	Fatty Acid Content of Fats and Oils	13
3	Catalysis of a Triglyceride by Lipases	14
4	Reaction steps of triacylglycerol hydrolysis	14
5	Incorporation of Oleic Acid in Palm Olein by Enzymatic Acidolysis at different Amount of Molecular Sieve	46
6a	Effect of Mole Ratio of Palm Olein: Oleic Acid (1:1) on Fatty Acid Composition (% FAC), Iodine Value (IV), Free Fatty Acid (FFA), Monoacylglycerol (MAG), Diacylglycerol (DAG) and Triacylglycerol (TAG)	49
6b	Effect of Mole Ratio of Palm Olein: Oleic Acid (1:2) on Fatty Acid Composition (% FAC), Iodine Value (IV), Free Fatty Acid (FFA), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	50
6c	Effect of Mole Ratio of Palm Olein: Oleic Acid (1:3) on Fatty Acid Composition (% FAC), Iodine Value (IV), Free Fatty Acid (FFA), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	51
7	Incorporation of Oleic Acid in Palm Olein by Enzymatic Acidolysis at different concentration of substrates	52
8a	Effect of Lipase Loading (<i>T. Lanoginosa</i>)1% (w/w) on Fatty Acid Composition (% FAC), Iodine Value (IV), Free Fatty Acid (FFA), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	57
8b	Effect of Lipase Loading (<i>T. Lanoginosa</i>) 5% (w/w) on Fatty Acid Composition (% FAC), Iodine Value (IV), Free Fatty Acid (FFA), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	58
8c	Effect of Lipase Loading (<i>T. Lanoginosa</i>) 10% (w/w) on Fatty Acid Composition (% FAC), Iodine Value (IV), Free Fatty Acid (FFA), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	59

9	Incorporation of Oleic Acid in Palm Olein by Enzymatic Acidolysis at different concentration of Lipase	60
10	Incorporation of Oleic Acid in Palm Olein by Enzymatic Acidolysis at different incubation Temperatures	63
11	Effect of Reaction Time on Fatty Acid Composition (% FAC), Iodine Value (IV), Free Fatty Acid (FFA), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	66
12	Catalytic Stability of Immobilised <i>T. Lanoginosa</i> Lipase in Repeated Batch Acidolysis of Palm Olein (i) Before Acidolysis and After (ii) Five and (iii) Ten Runs of Repeated Usage. Peaks marked X indicate the formation of Diacylglycerols	69
13	Fatty Acid Composition (% FAC), Free Fatty Acid (FFA), Monoacylglycerol (MAG) Diacylglycerol (DAG) and Triacylglycerol (TAG) in the Palm Olein Obtained From Using <i>T.Lanoginosa</i> Lipase After Ten Runs of Repeated Usage	70
14	Comparison of Oleic Acid (% FAC) and Palmitic Acid (% FAC) Content at Conditioning Process and After 10 Runs of Repeated Usage	71
15	Oleic Acid (18-1) and Palmitic Acid (16-0) content in Palm Olein catalysed by <i>T. Lanoginosa</i> and <i>Alcaligenes sp.</i> Lipase. (ii) is magnification of (i) from 0 to 10 h reaction time	75
16a	Effect of Temperature (50 °C) on Fatty Acid Composition (% FAC), Iodine Value (IV), Methyl Ester (ME), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	77
16b	Effect of Temperature (55 °C) on Fatty Acid Composition (% FAC), Iodine Value (IV), Methyl Ester (ME), Monoacylglycerol (MAG) and Triacylglycerol (TAG)	78
16c	Effect of Temperature (60 °C) on Fatty Acid Composition (% FAC), Iodine Value (IV), Methyl Ester (ME), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	79
17	Incorporation of Oleic Acid in Palm Olein by Enzymatic Transesterification at different incubation Temperatures	80
18a	Effect of Mole Ratio of Palm Olein: Methyl Oleate (1:1) on Fatty Acid Composition (% FAC), Iodine Value (IV), Methyl Ester (ME), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	82

18b	Effect of Mole Ratio of Palm Olein: Methyl Oleate (1:2) on Fatty Acid Composition (% FAC), Iodine Value (IV), Methyl Ester (ME), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	83
18c	Effect of Mole Ratio of Palm Olein: Methyl Oleate (1:3) on Fatty Acid Composition (% FAC), Iodine Value (IV), Methyl Ester (ME), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	84
19	Incorporation of Oleic Acid in Palm Olein by Enzymatic Transesterification at different concentration of substrates	85
20a	Effect of Lipase Loading (<i>T. Lanoginosa</i>) 1% (w/w) on Fatty Acid Composition (% FAC), Iodine Value (IV), Methyl Ester (ME), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	88
20b	Effect of Lipase Loading (<i>T. Lanoginosa</i>) 5% (w/w) on Fatty Acid Composition (% FAC), Iodine Value (IV), Methyl Ester (ME), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	89
20c	Effect of Lipase Loading (<i>T. Lanoginosa</i>) 10% (w/w) on Fatty Acid Composition (% FAC), Iodine Value (IV), Methyl Ester (ME), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	90
21	Incorporation of Oleic Acid in Palm Olein by Enzymatic Transesterification at different concentration of Lipase (<i>T. Lanoginosa</i>)	91
22	Effect of Reaction Time on Fatty Acid Composition (% FAC), Iodine Value (IV), Methyl Ester (ME), Monoacylglycerol (MAG) , Diacylglycerol (DAG) and Triacylglycerol (TAG)	95
23	Oleic Acid (18-1) and Palmitic Acid (16-0) content in Palm Olein catalysed by <i>T. Lanoginosa</i> and <i>Alcaligenes sp.</i> lipase	99
24	Solid Fat Content (SFC) of unreacted Palm Olein, Palm Olein:Oleic Acid yielded palm olein and Palm Olein:Methyl Oleate yielded palm olein at different Temperature	103
25	Melting Profile of Palm Olein and Interesterified Palm Olein	106
26	Cooling Profile of Palm Olein and Interesterified Palm Olein	106

LIST OF ABBREVIATIONS

12:0	lauric acid
14:0	myristic acid
16:0	palmitic acid
16:1	palmitoleic acid
18:0	stearic acid
18:1	oleic acid
18:2	linoleic acid
18:3	linolenic acid
AOCS	American Oil Chemist Society
area%	area percent
C46	mainly PMP
C48	mainly PPP
C50	mainly POP/PPO
C52	mainly POO and PLO
C54	mainly OOO
DAG	diacylglycerol
DSC	Differential scanning calorimetry
FA	Fatty acid
FAC	fatty acids content
FFA	free fatty acid
GC	gas chromatography
h	hour
HPLC	High Performance Liquid Chromatography

IV	iodine value
MAG	monoacylglycerol
ME	methyl ester
mins	minutes
MLP	1-myristoyl-2-linoleoyl-palmitoyl glycerol
MO	methyl oleate
mole %	mole percent
MPOB	Malaysian Palm Oil Board
MUFA	monounsaturated fatty acids
NMR	Nuclear Magnetic Resonance
OA	oleic acid
OLL	1-oleoyl-dilinoleoyl glycerol
OLO	1,3-dioleoyl-linoleoyl glycerol
OOO	tripalmitin
PLL	1-palmitoyl-dilinoleoyl glycerol
PLO	palmito-oleolinolein
PLP	1,3-dipalmitoyl-2-linoleoyl glycerol
PMP	myristodipalmitin
PO	Palm Oil
POO	dioleopalmitin
POo	palm olein
POP	dipalmitolein
POS	1-palmitoyl-2-oleoyl-stearoyl glycerol
PPP	tripalmitin

PPS	1,2-dipalmitoyl-stearoyl glycerol
PUFA	polyunsaturated fatty acids
RBD	refined bleached deodorized
SFC	solid fat content
SMP	slip melting point
SOO	1-stearoyl-dioleoyl glycerol
SOS	1,3 distearoyl-2-oleoyl glycerol
sp.	Species
SPD	Short Path Distillation
<i>T. Lanuginosa</i>	<i>Thermomyces Lanuginosa</i>
TAG	triacylglycerol
w/w	weight/weight

CHAPTER 1

INTRODUCTION

Palm oil is derived from the mesocarp of oil palm fruit, *Elaeis guineensis*, which originates from West Africa. In Malaysia, high yielding hybrid of Dura x Pisifera or Tenera is most commonly cultivated (Pantzaris, 1997). In the year of 2005, palm oil is the world's most widely produced (24% or 33.326 million tonnes) and consumed edible oil (21 million tonnes), slightly more than soybean oil (25.3% or 33.287 million tonnes), with Malaysia being the largest producer (45 %) and exporter (51.1 %) among palm oil output (MPOB, Oil World Annual, 1999-2005; Oil World Weekly, 16 Dec 2005; BCB, 2004). According to a forecast by Oil World (1994), the world production of palm oil projected to reach 25 million tones by the year 2008, but the numbers had exceeded, the projection showing the enormous potential in the global market. One of the challenges for the oil palm industry is to widen the application of palm oil.

Palm olein is the more liquid fraction obtained from palm oil fractionation after crystallization at a controlled temperature (Pantzaris, 1997). The co-product of the fractionation process is palm stearin, which is generally cheaper fraction used in shortening, margarine, and vanaspati manufacture (Pritchard 1969; Berger 1980; de Vries 1984; Idris *et al.* 1989), ice-cream manufacturing (Berger 1980), cocoa-butter substitute (Godin and Spensley, 1971). The physical characteristics of palm olein differ significantly from those of palm oil. Its fatty acids (FA) composition is 0.9-1.4% myristic acid, 37.9-41.7% palmitic acid, 4.0-4.8% stearic acid, 40.7-43.9% oleic acid, and 10.4-13.4% linoleic acid (Tan and Oh, 1981) while its TAG are

mainly of C48 (1.3-4.0 mol%), C50 (37.7-45.4 mol%), C52 (43.3-51.3 mol%), C54 (7.0-12.6 mol%) and absence of C46 (Tan and Oh, 1981). With a narrow range of slip melting point (SMP) ranging between 19.0 and 23.0 °C (Timms, 1985), palm olein is a natural liquid oil at ambient temperature, and is widely consumed as cooking and frying oil (Pritchard, 1969).

However, with its composition being prone to clouding, palm olein alone cannot withstand low temperature storage and exportation to temperate countries. Although technically there is nothing wrong with the oil quality, consumers tend to perceive cloudy oil as deteriorated product due to its unattractive appearance. Therefore, the retail market demands clear oils.

The fatty acid composition of edible oils (Hilditch, 1964) plays an important role in shelf life, nutrition and health. Oleic acid can be beneficial as monounsaturated fatty acid (MUFA) in reduction of low density lipoprotein (LDL) cholesterol without affecting the levels of high density lipoprotein (HDL) (Mattson and Grundy, 1985). Meanwhile, excessive saturated fatty acids diet, such as coconut oil (Ng *et al.*, 1991), long chain saturated fatty acids (LSFA) diets (Kritchevsky *et al.*, 1971), are observed to increase serum LDL cholesterol level, and contribute to atherosclerosis and carcinogenesis (Kubow, 1990), respectively.

There is considerable potential for direct competition in liquid oil market and higher nutritional value of palm olein if its physical and chemical properties can be modified. Processes such as double fractionation (Akaike, 1985), blending with